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Table 1. MR options for imaging post-stroke recovery

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Introduction

Thrombolysis and endovascular interventions have revolutionised stroke treatment, but many patients are excluded from such therapies, and residual disability is common.[1] Emerging approaches to enhance post-stroke brain repair may have no time constraints and are applicable to most stroke patients. Novel interventions to enhance brain repair include electromagnetic or robotic techniques, brain-computer interface, and restorative cell-based and pharmacological therapies.[2-4] A major impediment to translation to patient care, however, is the lack of robust *in vivo* techniques to monitor the effects of such interventions in humans.[3]

Non-invasive imaging of the human brain for multi-parametric *in vivo* monitoring of post-stroke recovery presents challenges. The clinical application of certain techniques such as positron emission tomography (PET) is frequently restricted by radiation exposure, limited resolution, high cost, or difficult access.[5-7] Magnetic resonance (MR) imaging (MRI) on the other hand is accessible, non-invasive, safe and versatile with high resolution, making this an ideal modality for multi-parametric *in vivo* monitoring of stroke recovery. This review will concentrate on MRI markers of stroke recovery in experimental models and, where available, in humans (Table 1).

Biological substrates of neural repair

Angiogenesis

The peri-infarct cortex is a unique neurovascular niche, within which angiogenesis is closely and causally linked to neurogenesis through vascular growth factors and chemokines.[2] Together with parenchymal astrocytes, angiogenic vessels facilitate synaptogenesis and

axonal sprouting.[2] Angiogenesis stimulated by cell-based or pharmacological interventions correlates with improved behavioural outcome.[2] In rodents, capillary sprouting at the ischemic boundary leads to new vessel development between 2 and 28 days.[8] Angiogenesis has been observed in the ischemic penumbra of humans 3 to 4 days after stroke, and higher cerebral blood vessel density has been associated with improved survival.[9] Angiogenic vessels are permeable during the early stages of development and become less leaky as they mature, potentially allowing new vessels to be distinguished by imaging techniques.[10]

Neurogenesis

In experimental stroke, focal ischemia increases neurogenesis in distinct parts of the ipsilateral subependymal zone (SEZ), and neuroblasts migrate from the SEZ to the ischemic boundary regions, where they exhibit phenotypes of mature neurons (Figure1).[11-13] It has been shown in rat models that migration of immature cells from the SEZ occurs over the first 2 weeks, but can be sustained for several months.[14] Neurogenesis after stroke has been associated with functional recovery in mice.[15] In humans, post-mortem studies have demonstrated active proliferation of astrocytes, neuroblasts, migrating neuroblasts and immature neurons in the ipsilateral SEZ at 10 days after stroke.[16-18] Cells expressing markers of new-born neurons have been detected in the ischemic penumbra of humans, where they cluster around blood vessels.[16, 19]

Axonal remodeling

It has been shown in rats that neurons in the surviving peri-infarct cortex are induced to express a growth-associated genetic profile that enhances axonal sprouting and mediates the formation of new connections.[20] In experimental stroke, axonal sprouting occurs in a growth-permissive zone outside the glial scar within the peri-infarct cortex, and also up to several

millimetres away from the infarct.[21] Significantly increased axonal connections, originating from the peri-infarct motor cortex, have been demonstrated during recovery in rodent and primate models, correlating well with behavioural outcome.[22, 23]

Imaging of Biological Substrates

Angiogenesis

Although direct depiction of microvessels with MR angiography is not feasible due to its relatively low spatial resolution, there are many MRI parameters that can be used to monitor angiogenesis and vascular remodeling after stroke indirectly (Table 1).[24, 25]

Hemodynamic parameters affected by angiogenesis, such as cerebral blood volume (CBV) and cerebral blood flow (CBF), can be measured with perfusion techniques such as dynamic susceptibility contrast-enhanced (DSC-) MRI or arterial spin labelling (ASL).[25] DSC-MRI calculates cerebral hemodynamic parameters from the time course of signal changes induced by the first passage of an intravenously injected paramagnetic contrast agent.[26] DSC-MRI has demonstrated significantly increased CBV in the recovering ipsilesional cortex of rodent stroke models, which correlated well with increased vascular density on histology.[27, 28] The ASL method applies radiofrequency pulses to alter the magnetisation of water protons in arterial blood, thereby generating an endogenous intravascular tracer.[29] Using ASL, a significantly enhanced CBF has been demonstrated in the perilesional cortex in rat models, that correlated with vascular density.[28, 30]

The low specificity of DSC-MRI or ASL for angiogenesis may be overcome by steady state susceptibility contrast-enhanced (SSCE)-MRI, which allows estimation of blood volume,

vessel size, and microvessel density.[25] The ratio of changes in gradient-echo to spin-echo relaxation rate ($\Delta R_2^*/\Delta R_2$) induced by a high-molecular weight intravascular contrast agent provides an estimate of average vessel size after accounting for echo time, contrast concentration, and the main magnetic field.[31] The ratio $Q \equiv \Delta R_2/(\Delta R_2^*)^{2/3}$ characterises microvascular density (MVD).[32] In a rat model of transient cerebral ischemia, SSCE-MRI showed decrease in vascular density and increase in vessel size due to strengthened collateral circulation in the re-perfused cortex at days 1 and 3, confirmed on histology, followed by an increase in vascular density likely attributable to angiogenesis at days 14 and 21.[33]

Increased permeability of the blood-brain-barrier around newly formed vessels can be assessed in humans and animal models with dynamic contrast-enhanced (DCE-) MRI, which measures the time-course of contrast-induced changes in the T_1 relaxation time constant due to contrast agent extravasation into tissue.[34] The blood-brain-barrier transfer constant, K_i, can be estimated by locally assessing the dynamics of the signal intensity of a time-series of T_1-weighted MR images with tracer kinetic models.[35] In a rat stroke model, K_i in the ischemic boundary correlated with histologically confirmed angiogenesis after cell-based treatment.[27] The increase of K_i, peaking at 2-3 weeks, co-localized with elevated CBF and CBV at 6 weeks after cell therapy. In another study, K_i elevation was detected 1-3 weeks after stroke in sildenafil treated animals compared with 1-6 weeks in controls, suggesting that therapy accelerates angiogenesis and this is measurable with imaging.[36]

Newly developed vasculature rich in venous blood can be detected with blood oxygenation level-dependent (BOLD) MRI.[36] This exploits the local magnetic field disturbances induced by the relatively high magnetic susceptibility of deoxygenated haemoglobin, which provides T_2 contrast on spin echo images and a larger T_2^* contrast on gradient echo images.[37]

Susceptibility weighted imaging (SWI), a variant of T_2* weighted MRI that includes phase information, can further enhance the contrast of the areas with increased magnetic susceptibility.[24, 38] In a rat model of stroke, perilesional angiogenesis was successfully identified on T_2* and SWI maps.[36] These techniques also detected an accelerated angiogenetic profile with sildenafil, and regions exhibiting T_2* shortening spatially matched with those with elevated K_i.[36] T_2* shortening in the ischemic hemisphere spatiotemporally corresponded with increased CBF on ASL in rats.[30]

MRI can be used to measure haemodynamic parameters, microvascular density, blood-brain barrier permeability, and deoxygenated haemoglobin, all of which are potential markers of angiogenesis and vascular remodeling. However, MRI is not without limitations and MRI techniques differ in their availability and suitability for clinical use. MRI measurement algorithms are also based on relatively basic biophysical and mathematical models which may be inaccurate under complex or altered conditions seen with stroke.[25]

DSC-MRI is arguably the most widely available perfusion MRI technique in the clinical setting, but DSC-MRI methods are not fully quantitative, and thus provide information with regard to the *relative* CBF and CBV.[39] BOLD methods are also widely available and easily implemented but are again able to provide only a *qualitative* assessment of deoxyhaemoglobin concentration or T2*. ASL methods are arguably more quantitative and in recent years have become increasingly available, although most ASL methods do not provide a measure of CBV.[39] Both DSC-MRI and ASL are sensitive to errors arising from an increase in arterial transit time to the peri-infarct tissue, and DSC-MRI perfusion values can also be confounded by dispersion of the tracer input function or T1 changes from blood brain barrier disruption.[39] In addition, while ASL and DSC-MRI offer good sensitivity to perfusion

changes during stroke recovery, alterations in CBF and CBV may reflect vasodilation or metabolic changes (Figure 2), so these perfusion measures may not offer a high specificity for angiogenesis per se. Similarly, an increase in K_i (from DCE-MRI) or a shortening of T_2* may arise because of other vascular pathology.[25] SSCE-MRI measures of microvascular density and vessel size arguably offer the greatest specificity for angiogenesis, but currently suffer from limited availability, especially within the clinical setting.

Neurogenesis

There are inherent limitations of resolution and sensitivity that preclude the direct visualization of endogenous neurogenesis. The unique metabolic properties of newly generated cells, however, can serve as surrogate markers. Magnetic resonance spectroscopy (MRS) potentially allows the *in vivo* measurement of marker compounds associated with different cell types. The metabolites most frequently detected in the human brain include choline, creatine, glutamate, glutamine, lactate, myo-inositol (mI), and N-acetyl aspartate (NAA), but there are at least 25 other less common metabolites that can be assessed by MRS.[40] Proton (^{1}H) MRS offers better sensitivity compared to other nuclei (phosphorus or carbon) and is more likely to detect minor changes in metabolites.[40]

In vitro nuclear magnetic resonance (NMR) spectroscopy, a correlate of *in vivo* MRS, can identify cultured neurons, glial and meningeal cells by their metabolic properties.[41] Cultured neural stem cells exhibit high levels of choline and mI, and low concentrations of creatine, a profile different from embryonic stem cells or mature cells of the central nervous system.[42] A spectroscopic peak at 1.28 parts per million (ppm), found in cultured cells and the rat and human hippocampi, has been reported as being unique to neural progenitor cells, although this specificity has subsequently been questioned.[43-47]

A variety of metabolites in the peri-lesion area can inform on cellular repair mechanisms. Both single- and multiple-voxel MRS techniques have been used to non-invasively measure *in vivo* alterations of metabolite profile during stroke recovery in both human studies and in animal models. $^{1}H/^{13}C$ MRS showed significant decrease in NAA, choline, and glutamate/glutamine turnover with increased glutamine and lactate at 24 hours in the perilesional areas of the rat brain, followed by normalization of NAA, choline, glutamine, and glutamate/glutamine turnover at 3 weeks, paralleling neurological improvement.[48] These alterations in the metabolite profile indicate substantial recovery of the initially impaired neural and glial functioning in the lesion border zone over time.[48] In humans, acute stroke is associated with elevated lactate and reduced NAA in the infarct zone, whereas the peri-infarct regions are characterised by lactate levels similar to the core but with NAA concentrations significantly higher than inside the infarct.[49, 50] In a longitudinal multi-voxel MRS study of 51 stroke patients, NAA concentrations fell both in infarcted and ipsilesional normal tissue over the first 2 weeks, and remained lower than baseline at 3 months, indicating neuronal loss.[51] A biphasic lactate peak was thought to represent initial tissue hypoxia followed by infiltration by inflammatory cells with a high rate of anaerobic glycolysis.[51] In another study, higher mI concentrations in the spared ipsi- and contra-lesional primary motor cortices of chronic stroke patients were thought to reflect astrocyte mediated post-stroke plasticity rather than gliosis.[52]

MRI contrast labelling offers an alternative approach for *in vivo* imaging of cellular elements during recovery.[5, 53, 54]Animal studies have employed superparamagnetic (iron oxide-based) particles and paramagnetic (gadolinium or manganese-based) agents, some of which are approved for human use.[5] Paramagnetic agents generate a hyperintense signal on T_1-weighted MRI, whereas cells harbouring even a single micron-sized iron oxide particle can be seen as

hypointense spots on T_2 and T_2*-weighted MRI, enabling precise anatomical localization and tracking of cell migration.[55] Neural stem cells labelled *in vitro* have successfully been detected *in vivo* in animal stroke models by means of MRI following transplantation (Figure 1).[5, 56, 57] A patient receiving an intravenous injection of iron-oxide microbead-labeled bone marrow stem cells 2 weeks after an ischemic stroke developed hypointensity compatible with iron (stem cell) deposition in the infarct, as detected with T2* MRI 4 days after the injection.[58] Animal studies also indicate that endogenous neuronal stem/progenitor cells can also be labelled using cerebroventricular injections of MRI contrast agents, but their application in humans may be limited.[55] Labelling can alter cellular functional characteristics, and may not allow reliable differentiation between viable and dead cells; and uptake of contrast by other cell types such as phagocytes cannot be excluded.[5, 53, 57, 59]

MRI approaches to detect cellular changes during stroke recovery include the identification of progenitor cells by their unique metabolic profile or by labeling with contrast agents. (^{1}H) MRS is available as an option on many commercial (human) MRI scanners, although more interactive optimisation is often required than is usual for MRI scanning, and considerable local expertise is necessary to ensure high quality data. MRS has low sensitivity, which is a major limitation if there are very few cells to detect, as is the case in the peri-lesion area harbouring sparsely seeded neural progenitors. The low sensitivity also means that large imaging voxels are required, which leads to partial volume effects and makes longitudinal follow-up harder as the relative concentrations of different components within a voxel may change over time. Furthermore, there is no one to one mapping of metabolites to cell types, which affects specificity.

Axonal remodeling and cortical changes

Diffusion tensor imaging (DTI) delineates anatomical connectivity of white matter pathways and can detect tract disruption.[60] DTI provides two scalar measures, the apparent diffusion coefficient (ADC) and fractional anisotropy (FA), which characterize the magnitude of water diffusion and the degree of anisotropy for each voxel (Figure 3).[24] In addition, careful interpretation of axial (parallel to the long axis of the fiber) and radial (perpendicular) diffusivity provides measures of axonal and myelination status, respectively.[24, 61]

Experimental stroke in rats has been characterized by decreased FA in perilesional areas at 3 days, followed by return to control values after 9 weeks.[62] In rat models of stroke, treatment with neural progenitor cells, sildenafil or erythropoietin has been associated with increased elevation of FA in peri-infarct areas at 5-7 weeks, which has correlated with reorganization of axons and myelin on histology, and with improved functional outcome.[30, 63, 64] In humans, a decrease in FA of the ipsilesional corticospinal tract has been detected during the first 3 months following ischemic stroke.[65] Early fiber tract degeneration has paralleled impaired recovery,[66] and predicted long-term deficits.[65] During a period of up to a few years after stroke, human studies (including some using specific interventions such as intonation-based speech therapy or brain-computer interface training)[67, 68] have observed increasing FA, connectivity and fiber number in lesioned and non-lesioned white matter, correlating with better functional outcome.[67-71] DTI evaluation of motor tract integrity in chronic stroke patients can predict motor function and behavioural gains from robotic therapy, motor practice, transcranial direct current or epidural motor cortex stimulation.[72-77]

DTI methods incorporating a single tensor fit to the diffusion at each voxel show an anomalous overall lowering of FA where white matter fiber tracts cross, despite the presence

of highly oriented tissue.[24] Newer q-space based and High Angular Resolution Diffusion Imaging (HARDI) techniques (e.g. diffusion spectrum imaging (DSI), q-ball, and persistent angular structure MRI (PAS-MRI)) provide model-independent analysis to obtain multiple tensors per voxel and thereby extract information on complex tissue structure including crossing fiber tracts.[60, 78-80] These techniques are particularly relevant in detecting randomly oriented crossing axons during early-stage axonal remodeling in stroke recovery.[60] In addition, changes in axonal density associated with stroke recovery can be measured using MRI diffusion entropy, a technique not dependent on axonal orientation.[60]

Manganese-enhanced MRI (MEMRI) is a novel tool for *in vivo* assessment of neuronal networks.[81] MEMRI is based on the detection of manganese (Mn^{2+}), which enters active neurons and is transported axonally and trans-synaptically.[81] Paramagnetic manganese shortens the T_1 relaxation time and increases signal intensity on T_1-weighted MRI. In a rat stroke model, decreased manganese enhancement in the ipsilateral sensorimotor network at 2 weeks after stroke was followed by enhanced connectivity on MEMRI and histology at 10 weeks, which correlated with behavioural recovery.[82]

MRI also allows the measurement of cortical thickness and gray matter density or volume.[83, 84] In chronic stroke patients, atrophy of gray matter in remote brain regions (likely a consequence of impaired connectivity) predicted a lesser extent of motor improvement from subsequent constraint-induced movement therapy or epidural motor cortex stimulation.[72, 85] Increases in gray matter have been demonstrated in compensatory brain regions of chronic stroke patients,[86] and following constraint-induced movement therapy in parallel with functional improvements.[87] The mechanism of this increase in gray matter is likely to be multifactorial involving both neural and non-neural elements.[86]

In summary, DTI and MEMRI are potent *in vivo* techniques to study white matter structure, and MRI also provides tools to measure dimensions of gray matter. DTI is available either as standard or as an option on most commercial MRI scanners, which also routinely provide volumetric T1-weighted measurements. However there are potential limitations. The interpretation of tractography data requires experience and knowledge of white matter anatomy. Complex analytic methods are computationally demanding, and require longer processing times.[88] Several tractography algorithms have been described, but there is no consensus on the best method to use. Only few studies have validated tractography with dissection or histology, and knowledge on the relationship of MR measures and specific axonal events is limited. DTI cannot differentiate anterograde from retrograde connections or determine whether a pathway is functional.[88] Manganese is neurotoxic at high concentrations or after chronic exposure, which limits MEMRI-based neuronal tracing in human studies.[89]

General considerations for human application and future directions

Non-invasive MRI biomarkers may provide deeper insights into specific neural events underlying stroke recovery. Furthermore, they may prove helpful to estimate prognosis, and identify patients who may benefit from specific interventions.[90, 91] The use of MRI biomarkers as surrogate end points may facilitate the screening of novel therapeutic interventions prior to large clinical trials. Considerations for surrogate markers should include feasibility of translation from animal to human studies, causal role in the disease process, and capacity to capture the net effect of therapy on clinical outcome. [91] MRI may not be the best brain-mapping tool for every aspect of stroke recovery: PET has a higher potential to characterize regional brain metabolism, and receptor and transporter distribution and

density;[6] transcranial magnetic stimulation (TMS) has advantages in evaluating the physiological properties of neural populations;[92] whereas magnetoencephalograpy (MEG) offers superior temporal resolution.[93]

The use of MRI to monitor recovery in humans presents additional challenges. There are well-known contraindications to MRI, and the administration of contrast agents may cause complications, particularly if given on multiple occasions.[94, 95] The most feasible clinical research protocols for the study of large patient populations in a longitudinal fashion would employ non-contrast techniques that are available on current scanner platforms (Table 1), with keeping in mind that sessions longer than 60 minutes are unlikely to be easily tolerated by most stroke patients. Imaging biomarkers are time dependent: some may be most useful to monitor recovery early after stroke (e.g. CBF),[25] whereas others may have limited value in the very early phase (e.g. FA may be transiently elevated on DTI as a consequence of edema).[96] The selection of regions of interest (ROI) for quantitative MRS, perfusion, or diffusion measurements requires hypotheses about the areas likely to be involved in post-stroke recovery, and focal abnormalities are often much easier to interpret if data from presumed normal tissue in the contralesional hemisphere are available for comparison.[40] It is also advisable that studies for research purposes use age-, gender-, and anatomically matched data from control subjects for comparison.[40]

Developments in MRI technology such as higher field strengths and faster MR acquisition methods will shorten scan times and make complex measurements more feasible. Further advantages of high field include improved spectral resolution, allowing the detection of more compounds with MRS,[40] and direct visualization of brain structures such as specific major fibre bundles.[97] However, high-field MRI is also associated with more prominent artefacts.[98]

Molecular imaging is another promising approach which may in future become more readily available. This may allow the monitoring of angiogenesis using paramagnetic contrast agents targeted against endothelial integrin $\alpha_v\beta_3$.[99, 100] Molecular MRI techniques for tracking cellular elements include imaging reporter genes (either with or without exogenous contrast administration) or contrast agent linked to receptor-specific antibodies.[5, 53] Contrast materials based on a heteronuclear (e.g. ^{19}F or ^{13}C) approach, or 'responsive' agents that change contrast due to their altered magnetic properties (e.g. relaxation or chemical shift) in response to dynamic changes in physiological, enzymatic and other metabolic properties are other potential advances.[53]

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Figure legend

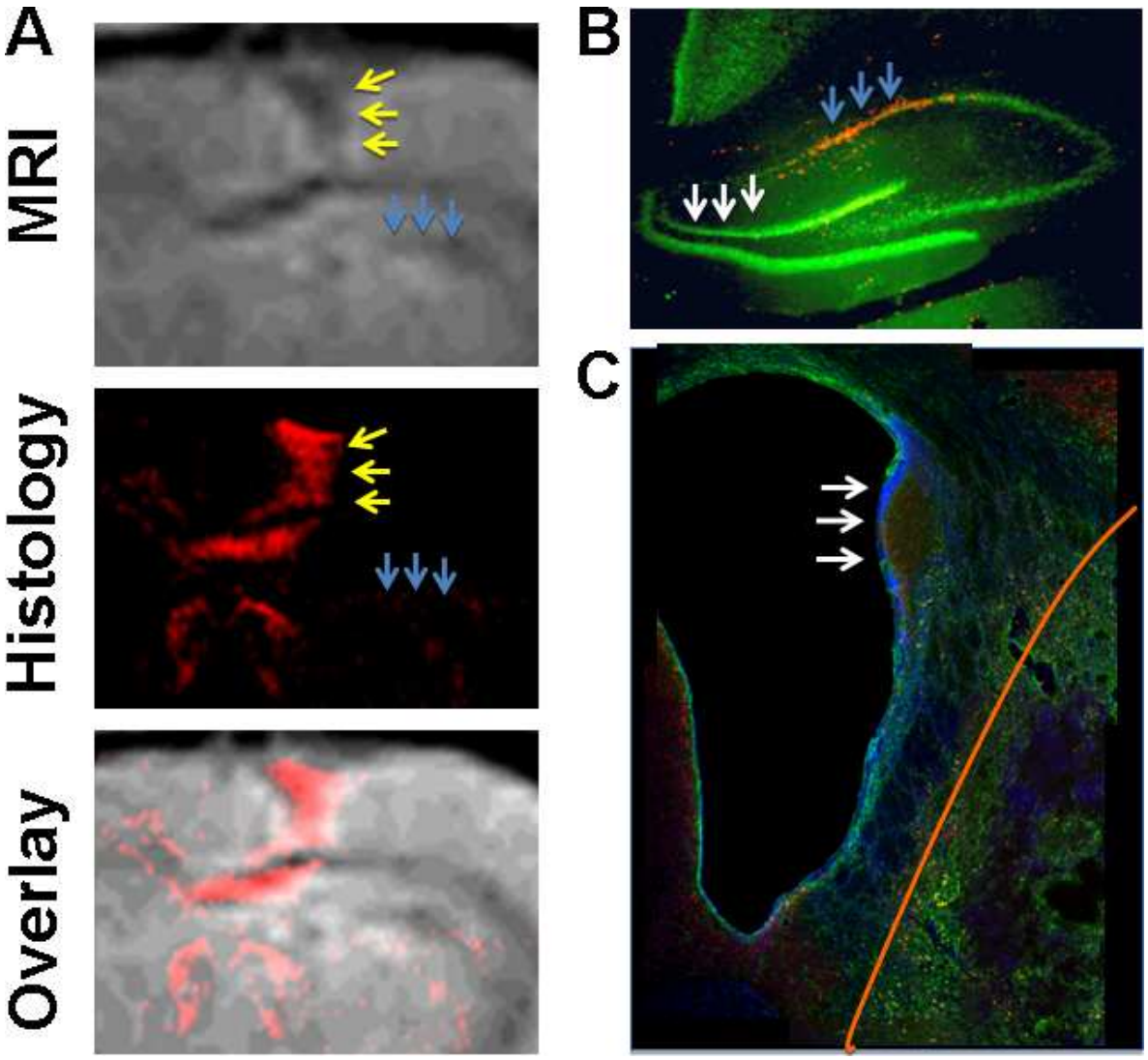

Figure 1. Imaging of transplanted and endogenous neural stem cells (NSCs)

A. Mouse neural stem cells labeled with a bimodal gadolinium-rhodamine (GRID) contrast agent can be detected both by MRI and fluorescence histology. The overlay of both images confirms good correspondence between the areas of transplanted cells on both imaging modalities. The blue arrows indicate the area of CA1 with transplanted cells, whereas the yellow arrows point to the injection tract.

B. In rats with global ischaemic damage (15 minutes of 4 vessel-occlusion), transplanted NSCs (in red due to rhodamine chelate from contrast agent) migrate into the CA1 cell layer

of the damaged hippocampus (NeuN+ neurons marked in green), corresponding to the hippocampal region on the MR image (blue arrows). Detection of endogenous neurogenesis, however, constitutes a major challenge as only a small proportion of cells within the dentate gyrus (white arrows) are proliferating neural stem cells or newly generated neurons.

C. A similar challenge lies in the in vivo detection of neurogenesis in the sub-ependymal zone (SEZ, white arrows) next to the lateral ventricle. The SEZ is a very dense cellular region as can be seen here with DAPI (blue) labeling of every cell nucleus, delineating a thin line along the lateral ventricle. Although this zone is normally only a few cells deep, some parts of SEZ expand due to a stroke (area delineated with orange line), still remaining within approximately 100 μm in thickness. In vivo ventricular motion as well as the dramatic variation in tissue type will be major challenges in the detection metabolic changes within this specific region. However, cells migrating into the peri-infarct area (astrocytes stained with GFAP are visible here in green) potentially can affect the metabolite profile of this area, although again the numbers infiltrating this area are relatively small compared to the overall volume of the peri-infarct area. Neuronal cells (NeuN in red) are dramatically decreased even in the peri-infract area, and very little cell replacement occurs due to endogenous neurogenesis.

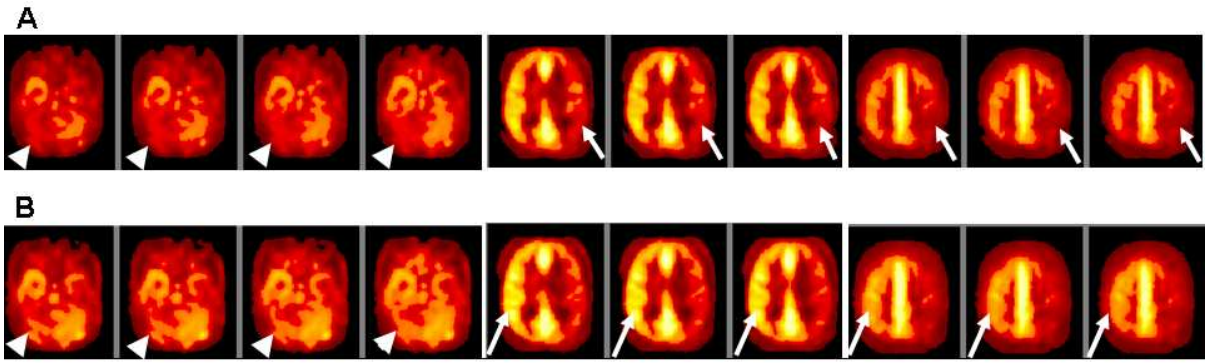

Figure 2. Group-average CBF maps using ASL in 6 patients with a cortical ischaemic stroke in the left middle cerebral artery territory. At 3 weeks after stroke (A) hypoperfusion in the affected hemisphere (arrows) and crossed-cerebellar diaschisis (arrowheads) is demonstrated. At 15 weeks perfusion (B, displayed with the same threshold as in A) is restored in the contralesional cerebellum (arrowheads), and is increased in the contralesional hemisphere (arrows).

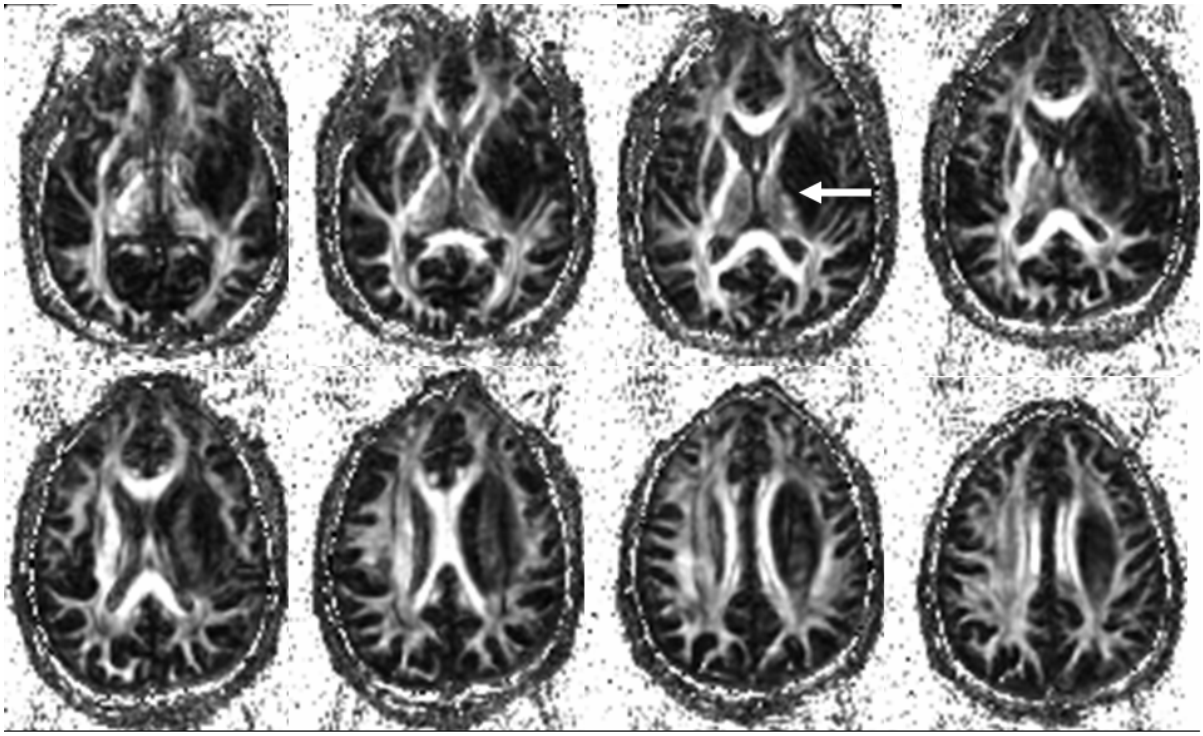

Figure 3. FA maps are easily obtainable on current scanners. FA values are decreased in the ipsilesional internal capsule (arrow) in a patient with left hemispheric ischaemic stroke.

Table 1. MR options for imaging post-stroke recovery

Technique	Need for exogenous contrast agent	Parameter measured	Availability on current scanner platform
Angiogenesis			
DSC-MRI	Yes	CBF, CBV	Yes
ASL	No	CBF	Yes
SSCE-MRI	Yes	Average vessel size, MVD	No
DCE-MRI	Yes	K_i	No
Gradient/spin echo BOLD	No	Deoxygenated haemoglobin	Yes
SWI	No	Deoxygenated haemoglobin	Yes
Cells			
MRS	No	Concentrations of metabolites	Yes
Cell labeling	Yes	Properties of contrast in cells	No
Axonal remodeling			
DTI	No	ADC, FA, Q-space based and HARDI measurements	Yes
MEMRI	Yes	Paramagnetic manganese in neural networks	No
Brain volume			
T1-weighted volumetry	No	Regional gray matter volume	Yes

ADC, apparent diffusion coefficient; ASL, arterial spin labelling; BOLD, blood oxygenation level-dependent imaging; CBF, cerebral blood flow; CBV, cerebral blood volume; DCE-MRI, dynamic contrast-enhanced MRI; DSC-MRI, dynamic susceptibility contrast-enhanced MRI; DTI, diffusion tensor imaging; FA, fractional anisotropy; HARDI, High Angular Resolution Diffusion Imaging; $K_{i,}$ blood brain barrier transfer constant; MEMRI, manganese-enhanced MRI; MRS, magnetic resonance spectroscopy; MVD, microvascular density; SSCE-MRI, steady state susceptibility contrast-enhanced MRI; SWI, susceptibility weighted imaging.